PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet)	
Applicant's or agent's file reference see form PCT/ISA/220	**FOR FURTHER ACTION** See paragraph 2 below
International application No. PCT/JP2005/004881	International filing date *(day/month/year)* 14.03.2005
Priority date *(day/month/year)* 12.03.2004	
International Patent Classification (IPC) or both national classification and IPC C12Q1/68	
Applicant CANON KABUSHIKI KAISHA	

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

European Patent Office - P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk - Pays Bas
Tel. +31 70 340 - 2040 Tx: 31 651 epo nl
Fax: +31 70 340 - 3016

Authorized Officer

Cornelis, K

Telephone No. +31 70 340-8957

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☒ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43*bis*.1 and 64.1) is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	2,4,12,14
	No:	Claims	1,3,5-11,13,15-23
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-24
Industrial applicability (IA)	Yes:	Claims	1-24
	No:	Claims	

2. Citations and explanations

see separate sheet

Reference is made to the following documents:

D1: WO 01/71041 A (MERGEN LTD; YU, ZAILIN; PENG, ZAOYUAN; HU, QIANJIN) 27 September 2001 (2001-09-27)

D2: WO 01/48242 A (MERGEN LTD; HU, QIANJIN; PENG, ZAOYUAN; YU, ZAILIN) 5 July 2001 (2001-07-05)

D3: WO 02/081743 A (HAMILL, BRENDAN) 17 October 2002 (2002-10-17)

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 NOVELTY

D1 discloses a method of detecting a nucleic acid comprising the steps of

preparing a single stranded nucleic acid with sequences to be detected and a complementary nucleic acid strand (page 3, line 12, page 13 lines 18-20)

preparing primers having one of the sequences to be detected, and immobilising them on an array

preparing a primer for elongating the complementary strand, which has a sequence located at the 3' end of the nucleic acid to be detected of the sense strand (page 14, lines 18-23)

performing PCR reactions using the sense and antisense strands as templates and using the primers which are immobilised and the primer for elongating the antisense strand

forming a hybridised product of a nucleic acid corresponding to the sense strand which has been elongated and amplified and is bound to the substrate and a nucleic acid corresponding to the antisense strand which is not bound to the substrate (pages 14 and 15).

detecting the sequence which has to be detected by detecting the hybridised product in the respective primer immobilised regions of the array (page 17 lines 18-23).

D1 uses this method also for multiple target nucleotides whereby multiple sequences on each can be detected, and uses labelled nucleotide monomers for the elongation.

Hence, in view of D1, independent claims 1, 3, 11 and 13 are not new.

Also claims 5-10, 16-21 are not new.

Furthermore, as D1 performs the method of claim 19, the apparatus of claim 21 is implicitly disclosed.

D1 also discloses a kit for detecting nucleic acid comprising a primer array, a PCR reaction reagent, and a nucleic acid detection reagent, suitable for performing the method according to claim 1, hence claim 23 is not new.

D2 discloses a method as in claims 11 and 13, 15-20 and the products of claims 21-23.

D3 discloses a method of detecting a nucleic acid comprising the steps of (pages 17-20)

preparing a single stranded nucleic acid with sequences to be detected and a complementary nucleic acid strand

preparing primers having one of the sequences to be detected, and immobilising them on an array

preparing a primer for elongating the complementary strand, which has a sequence located at the 3' end of the nucleic acid to be detected of the sense strand

performing PCR reactions using the sense and antisense strands as templates and using the primers which are immobilised and the primer for elongating the antisense strand

forming a hybridised product of a nucleic acid corresponding to the sense strand which has been elongated and amplified and is bound to the substrate and a nucleic acid corresponding to the antisense strand which is not bound to the substrate

detecting the sequence which has to be detected by detecting the hybridised product in the respective primer immobilised regions of the array .

D3 uses this method also for multiple target nucleotides whereby multiple sequences on each can be detected, and uses labelled nucleotide monomers for the elongation.

Hence, in view of D3, independent claims 1, 3, 11 and 13 are not new.

Also claims 5-10, 16-21 are not new.

Furthermore, as D3 performs the method of claim 19, the apparatus of claim 21, comprising a PCR reaction container and detection means is disclosed. Furthermore, D3 specifies that the PCR reaction container comprises a substrate with surface with immobilised nucleic acid, a reaction chamber and this substrate is transparent for the wavelength used for detection (a glass slide is transparent for several of the fluorophores) and implicitly a PCR unit involves a temperature control which does not interfere with the detection. Hence, also the subject matter of claim 22 is already disclosed in D3.

D3 also discloses a kit for detecting nucleic acid comprising a primer array, a PCR reaction reagent, and a nucleic acid detection reagent, suitable for performing the method according to claim 1, hence claim 23 is not new.

In summary, claims 1,3,5-11,13,16-23 are not new (Article 33(2) PCT).

2 INVENTIVE STEP

2.1 Claim 2

D3 is considered the closest prior art for the subject matter of claim 2 (see above) and claim 2 differs from D3 in that additional primers are added to the PCR solution. These primers are complimentary with the 5' of the sense strand. The technical effect of this difference is that an additional amplification of the sense strand in solution takes place, thus generating additional target strand for the primers on the array.

The problem solved by the subject matter of claim 2 may therefore be considered as the provision of a method which detects also small quantities of target DNA.

The solution of claim 2 is the use of primers complimentary with the 5' of the sense strand in the solution for PCR.

This solution cannot be considered as inventive as D3 already discloses primers which are identical to those on the array, and thus enable the elongation of (a part of) the sense strand, in the PCR solution. In addition the use of PCR in order to amplify the target sequences is well known in the art. Moreover it is not clear why amplifying the sense strand in the solution would be improving the detecting of small quantities of target, as it is the antisense strand which serves as a template for the primers which are attached to the array. Thus using a primer in the solution which enables the amplification of the sense strand in solution would have occurred to the person skilled in the art as one of several possible modifications to the method of claim 1.

2.2 Similarly, claims 4, 12 and 14 are not considered inventive (Article 33(3) PCT).